


IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of W. Robert Arathoon et al. Serial No.: not yet assigned	Group Art Unit: not yet assigned Examiner: not yet assigned
Filed: May 23, 2001 For: A METHOD FOR MAKING MULTISPECIFIC ANTIBODIES HAVING HETEROMULTIMERIC AND COMMON COMPONENTS	

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
 Washington, D.C. 20231

Sir:

Prior to review of the above-referenced application on the merits, please amend the application as follows:

In the Specification:

Please replace the paragraph beginning at page 13, line 26, with the following rewritten paragraph:

--(ii) recovering the multispecific antibody from the host cell culture.--

Please replace the paragraph beginning at page 17, line 16, with the following rewritten paragraph:

--Figs. 2A-2C. Fig. 2A diagrams a selection scheme for C_H3 heterodimer using phage display vector, pRA2. Phage displaying stable C_H3 heterodimers are captured using an antibody directed to the gD flag. Fig. 2B diagrams a dicistronic operon in which C_H3 expressed from a synthetic gene is co-

secreted with a second copy of C_H3 expressed from the natural gene (Ellison et al. Nucleic Acids Res. 10:4071-4079 (1982)) as a fusion protein with M13 gene III protein. The synthetic C_H3 gene is preceded by a sequence encoding a peptide derived from herpes simplex virus glycoprotein D (gD flag, Lasky, L. A. and Dowbenko, D. J. (1984) DNA 3:23-29; Berman, P. W. *et al.*, (1985) Science 227:1490-1492 and a cleavage (G) site for the site-specific protease, Genenase I (Carter, P. *et al.* (1989) Proteins: Structure, Function and Genetics 6:240-248). Fig. 2C is the nucleic acid sequence of the dicistronic operon (SEQ ID NO:13) of Fig. 2B in which the residues in the translated C_H3 genes are numbered according to the Eu system of Kabat et al. In Sequences of Proteins of Immunological Interest, 5th ed. vol. 1, pp. 688-696, NIH, Bethesda, MD (1991). Protuberance mutation T366W is shown, as are the residues targeted for randomization in the natural C_H3 gene (366, 368, and 407).--

Please replace the paragraph beginning at page 95, line 29, with the following rewritten paragraph:

--A large human single chain Fv (scFv) antibody library (Vaughan *et al.* (1996), *supra*) was panned for antibodies specific for eleven antigens including Axl(human receptor tyrosine kinase ECD), GCSF-R (human granulocyte colony stimulating factor receptor ECD), IgE (murine IgE), IgE-R (human IgE receptor α -chain), MPL (human thrombopoietin receptor tyrosine kinase ECD), MusK (human muscle specific receptor tyrosine kinase ECD), NpoR (human orphan receptor NpoR ECD), Rse (human receptor tyrosine kinase, Rse, ECD), HER3 (human receptor tyrosine kinase HER3/c-erbB3 ECD), Ob-R (human leptin receptor ECD), and VEGF (human vascular endothelial growth factor) where ECD refers to the extracellular domain. The nucleotide sequence data for scFv fragments from populations of antibodies raised to each antigen was translated to derive corresponding protein sequences. The V_L sequences were then compared using the program "align" with the algorithm of Feng and Doolittle (1985, 1987, 1990) to calculate the percentage identity between all pairwise combinations of chains (Feng, D.F. and Doolittle, R.F. (1985) J. Mol. Evol. 21:112-123; Feng, D.F. and Doolittle, R.F. (1987) J. Mol. Evol. 25:351-360; and Feng, D.F. and Doolittle, R.F. (1990) Methods Enzymol. 183:375-387). The percent sequence identity results of each pairwise light chain amino acid sequence comparison were arranged in matrix format (see Table 6.1-6.15).--

Please amend the specification by renumbering pages 104-111 to be pages 116-123. Please further amend the specification by inserting after page 103 the attached Sequence Listing as pages 104-115.

In the Claims:

Please cancel claims 2-7, 10, 12-18, and 21-29 without prejudice to later prosecution. Please amend claims 1, 8, 9 and 19 as follows:

1. (Amended) A method of preparing a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain, wherein the first and second variable light chains have at least 80% amino acid sequence identity, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide and second polypeptide, and the first and second variable light chain, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the bispecific antibody from the host cell culture.

8. (Amended) The method of claim 1 wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

9. (Amended) The method of claim 8 wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

19. (Amended) A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain,

a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain, wherein the first and second variable light chains have at least 80% amino acid sequence identity, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain.

REMARKS

Formal Matters

Claims 1, 8, 9, 11, and 19-20 are pending in the application. Claims 2-7, 10, 12-17, and 21-29 have been canceled without prejudice to later prosecution. The claims were canceled in part to comply with a restriction requirement, and in part to focus the issues for prosecution in order to expedite allowance of the claims. Specifically, claims 12-18, 28, and 29 were withdrawn from consideration due to a restriction requirement.

Claims 1, 8, 9, 11, and 19-20 have been amended to more particularly point out and distinctly claim Applicants' invention. Support for the amendments is found throughout the specification, such as in the Definitions section beginning on page 19 of the specification, in the Examples, the Figures, and Table 6.1-6.15. For example, use of the term "antibody" to modify "variable heavy chain" and "variable light chain" is supported at page 11, lines 1-9; page 12, line 23 to page 13, line 2; page 16, lines 8-12; page 17, lines 6-14 and Fig. 1A-1C; page 19, lines 1-7 and Fig. 6; page 21, line 27 to page 22, line 24; and elsewhere throughout the specification. Use of the term "different binding domains" and the concept of the same or similar light chains functioning in different binding domains are supported at page 19, lines 16-18; page 22, lines 9-24; page 27, lines 24-25. No new matter is added by the amendments. Entry of the amendments is respectfully requested.

If the Examiner believes a telephone conference would expedite the prosecution of this application, the Examiner is invited to call the undersigned at the number indicated below.

This response is timely submitted with a transmittal letter during the pendency of parent application U.S. Serial No. 09/070,166. In the unlikely event that these documents are separated from this response or if it is deemed that this continuation application is not timely filed, Applicants hereby petition the Commissioner to file a petition for extension of time and authorize the Commissioner to charge our Deposit Account 07-0630 for any fees required or credits due for any extensions of time necessary to maintain the pendency of this application.

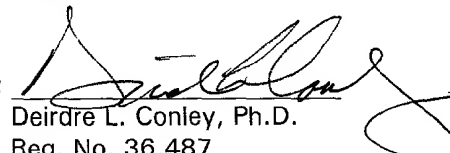
Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,

GENENTECH, INC.

Date: May 23, 2001

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PATENT TRADEMARK OFFICE

PC Docs #91142

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at page 13, line 26 has been amended as follows:

(ii) recovering the multispecific antibody from the host cell culture[;].

The paragraph beginning at page 17, line 16 has been amended as follows:

Figs. 2A-2C. Fig. 2A diagrams a selection scheme for C_H3 heterodimer using phage display vector, pRA2. Phage displaying stable C_H3 heterodimers are captured using an antibody directed to the gD flag. Fig. 2B diagrams a dicistronic operon in which C_H3 expressed from a synthetic gene is co-secreted with a second copy of C_H3 expressed from the natural gene (Ellison et al. Nucleic Acids Res. 10:4071-4079 (1982)) as a fusion protein with M13 gene III protein. The synthetic C_H3 gene is preceded by a sequence encoding a peptide derived from herpes simplex virus glycoprotein D (gD flag, Lasky, L. A. and Dowbenko, D. J. (1984) DNA 3:23-29; Berman, P. W. *et al.*, (1985) Science 227:1490-1492 and a cleavage (G) site for the site-specific protease, Genenase I (Carter, P. *et al.* (1989) Proteins: Structure, Function and Genetics 6:240-248). Fig. 2C is the nucleic acid sequence of the dicistronic operon (SEQ ID NO:[1] 13) of Fig. 2B in which the residues in the translated C_H3 genes are numbered according to the Eu system of Kabat et al. In Sequences of Proteins of Immunological Interest, 5th ed. vol. 1, pp. 688-696, NIH, Bethesda, MD (1991). Protuberance mutation T366W is shown, as are the residues targeted for randomization in the natural C_H3 gene (366, 368, and 407).

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Rse (human receptor tyrosine kinase, Rse, ECD), HER3 (human receptor tyrosine kinase HER3/c-erbB3 ECD), Ob-R (human leptin receptor ECD), and VEGF (human vascular endothelial growth factor) where ECD refers to the extracellular domain. The nucleotide sequence data for scFv fragments from populations of antibodies raised to each antigen was translated to derive corresponding protein sequences. The V_L sequences were then compared using the program "align" with the algorithm of Feng and Doolittle (1985, 1987, 1990) to calculate the percentage identity between all pairwise combinations of chains (Feng, D.F. and Doolittle, R.F. (1985) J. Mol. Evol. 21:112-123; Feng, D.F. and Doolittle, R.F. (1987) J. Mol. Evol. 25:351-360; and Feng, D.F. and Doolittle, R.F. (1990) Methods Enzymol. 183:375-387). The percent sequence identity results of each pairwise light chain amino acid sequence comparison were arranged in matrix format (see [Appendix] Table 6.1-6.15).

Pages 104-111 have been renumbered to be pages 116-123. The attached Sequence Listing has been inserted as pages 104-115.

In the Claims:

Claims 2-7, 10, 12-18 and 21-29 have been cancelled. Claims 1, 8, 9 and 19 have been amended as follows:

1. (Amended) A method of preparing a [multispecific] bispecific antibody comprising a first polypeptide and [at least one additional] a second polypeptide, wherein

[(a) the first polypeptide comprises a multimerization domain forming an interface positioned to interact with an interface of a multimerization domain of the additional polypeptide,

(b) the first and additional polypeptides each comprise a binding domain, the binding domain comprising a heavy chain and a light chain, wherein the variable light chains of the first and additional polypeptides comprise a common sequence, the method comprising the steps of:]

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain, wherein the first and second variable light chains have at least 80% amino acid sequence identity, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide and [additional] second polypeptide, and the first and second variable light chain, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the [multispecific] bispecific antibody from the host cell culture.

8. (Amended) The method of claim 1 wherein the first polypeptide and [additional] second polypeptide each comprise an antibody constant domain.

9. (Amended) The method of claim[s] 8 wherein the first polypeptide and [additional] second polypeptide each comprise an antibody constant domain [selected from the group consisting of a C_H3 domain and an IgG] from a C_H3 domain or from an IgG.

11. (Reiterated) The method of claim 1 wherein step (i) is preceded by a step wherein the nucleic acid encoding the first and additional polypeptide is introduced into the host cell.

19. (Amended) A host cell comprising nucleic acid encoding [the heteromultimer of claim 13] a

bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain, wherein the first and second variable light chains have at least 80% amino acid sequence identity, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain.

CLEAN SET OF ALL PENDING CLAIMS

1. A method of preparing a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain, wherein the first and second variable light chains have at least 80% amino acid sequence identity, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, the method comprising the steps of:

(i) culturing a host cell comprising nucleic acid encoding the first polypeptide and second polypeptide, and the first and second variable light chain, wherein the culturing is such that the nucleic acid is expressed; and

(ii) recovering the bispecific antibody from the host cell culture.

8. The method of claim 1 wherein the first polypeptide and second polypeptide each comprise an antibody constant domain.

9. The method of claim 8 wherein the first polypeptide and second polypeptide each comprise an antibody constant domain from a C_H3 domain or from an IgG.

11. The method of claim 1 wherein step (i) is preceded by a step wherein the nucleic acid encoding the first and additional polypeptide is introduced into the host cell.

19. A host cell comprising nucleic acid encoding a bispecific antibody comprising a first polypeptide and a second polypeptide, wherein

(a) the first polypeptide comprises a first multimerization domain that interacts with a multimerization domain of the second polypeptide,

(b) the first polypeptide and second polypeptide each comprise a different binding domain, a first binding domain comprising a first antibody variable heavy chain and a first antibody variable light chain, and a second binding domain comprising a second antibody variable heavy chain and a second antibody variable light chain, wherein the first and second variable light chains have at least 80% amino acid sequence identity, and

(c) the bispecific antibody is formed by the first variable light chain interacting with the first or second variable heavy chain in the first or second binding domain, and the second variable light chain interacting with the first or second variable heavy chain in the first or second binding domain.

20. The host cell of claim 19 wherein the host cell is a mammalian cell.